

Chronic Morbilliviral Encephalitis in a Young Striped Dolphin from Italy

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SUMMARY

A young male striped dolphin (*Stenella coeruleoalba*) found stranded lifeless in November 2009 was affected by a chronic non-suppurative encephalitis, characterized by prominent mononuclear cell perivascular cuffs, neuronal degeneration, microgliosis, neuronophagia and occasional presence of multinucleate syncytia. Immunohistochemical (IHC) and biomolecular (reverse transcription-polymerase chain reaction, RT-PCR) investigations for *Morbillivirus* were positive exclusively from the brain, but not from the cerebellum nor from any other tissue, with morbilliviral antigen being detected in neurons and, to a lesser extent, also in astrocytes. A low neutralizing antibody titer (1:10) against *Morbillivirus* (CDV) was also found, with no simultaneous presence of anti-*Brucella* antibodies in the blood serum from this animal.

We believe the present case is of interest since it represents the second morbilliviral encephalitis description in a striped dolphin found stranded on the Italian coastline, after over 10 years from previous reports of this lesion. Additional interest is provided by the peculiar neuro-histopathological, IHC and biomolecular features, with “antigenic” and “genomic” positivity being exclusively confined to the brain of this dolphin.

INTRODUCTION

BETWEEN the end of 2006 and the beginning of 2007, a *Morbillivirus* epidemic was reported in pilot whales (*Globicephala melas*) around the Strait of Gibraltar (Fernández and others 2008) and, in the following months, also in pilot whales and striped dolphins (*Stenella coeruleoalba*) along the Spanish Mediterranean coast (Raga and others 2008). Apart from its milder proportions, this mortality outbreak shares many similarities with the striped dolphin mass die-off that occurred throughout 1990-1992 in the Mediterranean Sea, which was caused by a newly discovered agent, named *Dolphin Morbillivirus* (DMV) (Domingo and others 1990, 1992, Barrett and others 1995, Kennedy 1998, Di Guardo and others 2005). More in detail, the DMV strain causing the above mortality episodes in pilot whales and striped dolphins shows a very close genetic relatedness to the DMV isolate responsible for the dramatic die-off which took place 15 years before in the same area (Fernández and others 2008, Raga and others 2008, Van Bresseem and others 2009). Furthermore, direct evidence of *Morbillivirus* infection has been recently reported in several striped dolphins, one pilot whale and one bottlenose dolphin (*Tursiops truncatus*) found stranded on the French Mediterranean coast (Keck and others 2010).

MATERIALS AND METHODS

On November 15 2009, a young male striped dolphin, measuring 112 cm in body length, was found stranded lifeless and in a very fresh preservation status on the Latium coast of Italy (just near Rome) and promptly submitted to *post mortem* examination. Samples of all animal's major organs, pulmonary and mesenteric lymph nodes were fixed in 10% neutral buffered formalin and submitted to detailed histopathological investigations as well as to immunohistochemistry (IHC) against *Morbillivirus*. This was carried out by means of a commercially available (VMRD Inc) mouse monoclonal antibody against *Canine Distemper Virus* (CDV) nucleoprotein (N) antigen that recognizes the same epitope from different *Morbillivirus* genus members, including DMV (Di Guardo and others 2010). Tissue extracts from the above organs were also submitted to a reverse transcription-polymerase chain reaction (RT-PCR) technique targeting a highly conserved N gene fragment of 287 base pairs (Frisk and others 1999). Finally, a virus neutralization assay detecting anti-*Morbillivirus* (CDV) antibodies and a serum agglutination test for anti-*Brucella* spp. antibodies were performed on the blood serum obtained from this dolphin.

RESULTS

Grossly, a moderate lung congestion associated with a mild liver degeneration and a chronic, diffuse catarrhal enteritis were observed, with many specimens of the cestode *Tetrabothrium forsteri* being also present in the intestinal lumen. Histopathology revealed a mild lymphoid interstitial pneumonia, along with a moderate, diffuse liver steatosis. The main lesion was a chronic, multifocal, non-suppurative encephalitis, characterized by prominent mononuclear cell (lymphohistiocytic and plasmacytic) perivascular cuffs (Fig 1), neuronal degeneration, microgliosis, neuronophagia and occasional presence of multinucleate syncytia, with these changes mostly affecting the cortical and subcortical grey matter. *Morbillivirus* IHC and RT-PCR were positive exclusively from the brain, but not from the cerebellum nor from any other tissue. More in detail, large amounts of viral antigen were detected in neurons as well as in their axons, dendrites, the surrounding neuropil and, to a lesser extent, also in astrocytes (Fig 2). Single, positively stained intranuclear inclusions could be occasionally observed in neurons. Additionally, a low neutralizing antibody titer (1:10) against *Morbillivirus* (CDV) was found, with no simultaneous presence of anti-*Brucella* antibodies in the blood serum from this animal.

DISCUSSION

Before the present case, direct evidence of *Morbillivirus* infection within the specific context of the 2006-2008 Mediterranean Sea epidemic has been reported, along the Italian coastline, only in another striped dolphin found stranded in June 2007 on the north-western coast of Sardinia (Appino and others 2008). In this respect, it should be also emphasized that a significant increase in striped dolphins' mortality was observed along the Ligurian Sea coast of Italy (neighbouring the French coastline) during the second half of 2007 (F. Garibaldi and W. Mignone, personal communication). Nevertheless, no IHC nor RT-PCR evidence of *Morbillivirus* infection was found in any of 8 striped dolphins found stranded on the Ligurian coastline from August to December 2007, 4 of which had anti-*Morbillivirus* (CDV) neutralizing antibodies in their sera. Furthermore, a multifocal, severe, non-suppurative meningo-encephalitis was observed in the remaining 4 dolphins, all seropositive to *Toxoplasma gondii* (and *Morbillivirus*-seronegative), 2 of which also showed IHC evidence of *T. gondii* cysts and zoites in their brain (Di Guardo and others 2010).

The case reported here is of additional concern, however, since IHC and RT-PCR evidence of *Morbillivirus* were exclusively limited to the brain, with the cerebellar and all other tissues from this animal appearing unaffected. Similar findings had been previously described after the acute phase of the 1990-1992 DMV epidemic in the Mediterranean Sea, during which several adult striped dolphins developed a chronic, non-suppurative encephalitis, with *Morbillivirus*-positive immunolabeling being observed only in the brain (and, occasionally, also in the cerebellum) from these animals, with no other tissue apparently harbouring DMV antigen (Domingo and others 1995). Differently from what above, however, the present case of chronic morbilliviral encephalitis occurred in a young dolphin, which raises the intriguing possibility that the brain lesions might have resulted from trans-placental infection. In this respect, there is only one available description - to

the best of our knowledge - of materno-faetal *Morbillivirus* (DMV) transmission among free-ranging cetaceans, which has been reported in a pilot whale foetus during the 2006-2007 die-off in Gibraltar and along the southern Spanish Mediterranean coast (Fernández and others 2008).

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Figures' Legend

- 1) **Figure 1:** Striped dolphin (*Stenella coeruleoalba*). Brain. Morbilliviral encephalitis. Prominent mononuclear cell (lympho-histiocytic and plasmacytic) perivascular cuffs are clearly visible in the subcortical grey matter. Haematoxylin and eosin (H&E) x 250.
- 2) **Figure 2:** Striped dolphin (*Stenella coeruleoalba*). Brain. Morbilliviral encephalitis. Large amounts of viral antigen are shown in subcortical neurons as well as in their axons, dendrites and the surrounding neuropil. Mayer's haematoxylin counterstain x 250.



